

PLANT DIVERSITY AND NITROGEN ADDITION ON BELOWGROUND  
BIODIVERSITY AND SOIL ORGANIC CARBON STORAGE IN BIOFUEL  
CROPPING SYSTEMS

by

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**DEFENSE COMMITTEE AND FINAL READING APPROVALS**

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Soil Organic Carbon Storage in Biofuel Cropping Systems

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## DEDICATION

To those that push themselves to greater heights, don't let the expectations of others be your anchor.

## ACKNOWLEDGMENTS

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## ABSTRACT

Bioenergy production may reduce the emission of CO<sub>2</sub> which contributes to climate change, particularly when management strategies are adopted that promote soil carbon (C) sequestration in bioenergy cropping systems. Planting perennial native grasses, such as switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman) may be used as a strategy to enhance soil C accumulation owing to their extensive root systems. Fertilizer use may further promote soil C sequestration, because of its positive impacts on plant production and soil C input. However, the influence of fertilizer addition on soil C accumulation is variable across bioenergy cropping systems, and fertilizer can negatively impact the environment. Increasing plant diversity may be used as a strategy to enhance soil C accumulation while augmenting other ecosystem properties such as soil biodiversity. The present study evaluates how inter- and intra-specific plant community diversity and N addition influence soil C storage and soil biodiversity. Soil was collected from a long-term (9 growing seasons) field experiment located at the Fermilab National Environmental Research Park in Illinois, USA. Treatments included [1] three cultivars of big bluestem and three cultivars of switchgrass cultivars grown in monoculture, [2] plant community diversity manipulated at both the species- and cultivar level, and [3] nitrogen (N) applied annually at two levels (0 and 67 kg ha<sup>-1</sup>). The soil at the site was dominated by C<sub>3</sub> grasses for 30 years before replacement with C<sub>4</sub> bioenergy grasses, which enabled quantification of plant-derived C accumulation owing to the natural difference in isotopic signature between C<sub>3</sub> and C<sub>4</sub> grasses. Soil

samples were analyzed for [1] soil C and its  $\delta^{13}\text{C}$  isotopic signature, and [2] nematode and soil bacterial diversity. Our results indicate that both plant diversity and N addition influence soil community structure but not soil C storage or soil nematode biodiversity. However, the addition of big bluestem to the plant species mixes enhanced plant-derived C storage. In summary, our findings suggest that plant species identity can control soil C accumulation in the years following land conversion, and that manipulating plant community structure in bioenergy cropping systems may have a greater positive impact on soil C accumulation than N fertilization.

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## THESIS

### Introduction

Atmospheric carbon dioxide (CO<sub>2</sub>) concentrations are increasing in response to anthropogenic activities, such as the burning of fossil fuels for energy (IPCC 2014). Because increased atmospheric CO<sub>2</sub> levels contribute to climate change, it is important to identify mechanisms by which we can reduce atmospheric CO<sub>2</sub> levels while satisfying society's energy needs (IPCC 2019). Biofuel production is one way by which we can reduce the emissions of fossil fuels and fulfill our energy demand. Mitigation of atmospheric CO<sub>2</sub> levels through the use of biofuels can be enhanced by growing biofuel crops that lead to an increase in soil C sequestration. Soils are the largest terrestrial carbon (C) pool (Scharlemann et al., 2014), and when soils accumulate more C, they contribute to reducing atmospheric CO<sub>2</sub> concentration, thereby mitigating climate change.

Soil C accumulation may be increased by converting Conservation Reserve Program (CRP) land from old fields dominated by non-native C<sub>3</sub> plants to 2<sup>nd</sup> generation biofuel production systems planted with native perennial grasses (Carriquiry et al., 2011; Adler et al., 2009). The CRP was established in the 1980s to reduce land degradation (USDA/FSA website). Large areas of cropland (e.g. 53,418 hectares in IL) were taken out of production and were converted to old fields dominated by non-native cool-season C<sub>3</sub> grasses. In some areas, these grasslands have since been converted to cellulosic bioenergy cropping systems comprised of native grasses such as switchgrass (*Panicum*

*virgatum* L.) and big bluestem (*Andropogon gerardii* Vitman). These species are promising candidates for this renewable energy strategy, because they compete with food production, and these native grasses stimulate soil C accumulation through their extensive root systems which stimulate soil C input and remain undisturbed even after aboveground biomass harvest (Giannoulis et al, 2016; Naik et al., 2010). However, the magnitude by which these cropping systems contribute to soil C accumulation depends strongly on management strategies that optimize soil C input through plant production and retention of this C in the soil (Rodrigues et al., 2017; Stewart et al., 2017; Kantola et al., 2017; Adkins et al., 2016; Robertson et al., 2011; Schmer et al., 2011).

Soil C storage can be increased through the application of nitrogen (N) fertilizers which increase biomass production (Frank, 2004; Geisseler and Scow, 2014), thus enhancing soil C input (Chatterjee et al., 2018; Thirukkumaran and Parkinson, 2002; Jung and Lal, 2011; Stewart et al., 2015). However, fertilization can lead to eutrophication of waterways (Anderson et al., 2018), and its production requires substantial energy input from fossil fuels that, from an energetic perspective, reduces the net benefit of growing biofuel crops from an energy perspective (Woods et al., 2010). Nitrogen addition may also reduce microbial biomass (Bradley et al., 2006), arbuscular mycorrhizal fungi (AMF) abundance (Treseder, 2004), and microbial and soil faunal biodiversity when applied at high concentrations (de Graaff et al., 2019; Fierer et al., 2012; Larson et al., 2018, Frey et al. 2014, Wei et al. 2012). Given that soil organisms regulate soil C and N dynamics as well as important ecosystem services, loss of soil biodiversity following N addition is a major environmental concern (McBratney et al., 2014; Wall, 2012). The scientific community identifies agricultural intensification,

including widespread use of fertilizer N inputs, as the greatest risk factor impacting belowground biodiversity (Orgiazzi et al., 2016). Thus, we must explore alternative management strategies that support a more environmentally sustainable production of bioenergy crops.

As an alternative to N addition, increasing plant species diversity may be used as a management option in cellulosic bioenergy cropping systems to enhance ecosystem functioning, including soil C accumulation (Morris et al., 2016). Greater rates of soil C sequestration with increased aboveground plant community diversity may be a consequence of diversity-induced increases in plant production and concomitant soil C inputs (Tilman et al., 2006; Lange et al., 2015; Morris et al., 2016). Alternatively, diversification of the chemical composition of root derived C inputs may promote soil C retention (Zhu et al., 2018). Increased aboveground plant community diversity can also enhance belowground ecosystem structure and functioning because of increased resource availability for the soil organismal community (Ito et al., 2015). For example, there have been reports that increased plant diversity increases belowground diversity of soil organisms, including bacteria and nematodes (Dick, 1992; Lange et al., 2015; Viketoft et al., 2009; Korboulewsky et al., 2016). These increases can promote resistance of soils to disturbance (Ekschmitt et al., 2001; Rodrigues et al., 2017; Stewart et al., 2017; Porazinska et al., 1999) and stabilization of soil C (Schimel & Schaeffer, 2012; Steinbeiss et al., 2008). However, it is uncertain how increasing inter- and intraspecific plant community diversity relative to increasing N fertilizer inputs impact soil C accumulation and soil organismal diversity in cellulosic bioenergy cropping systems.

Here, we ask how inter- and intra-specific plant diversity and N addition impact soil biodiversity and soil C storage. We hypothesize that increased plant community diversity will increase soil C accumulation and soil biodiversity, while N fertilizer will increase soil C accumulation and decrease soil biodiversity. Soil (0-15cm depth) was collected from a long-term (9 growing seasons) field experiment located at the Fermilab National Environmental Research Park in Illinois, USA. Treatments included a variety of big bluestem and switchgrass cultivars grown in monoculture, plant diversity manipulated at both the species- and cultivar-level, and nitrogen (N) applied at two levels (0 and 67 kg ha<sup>-1</sup>). The soil was dominated by C<sub>3</sub> grasses for 30 years before replacement with C<sub>4</sub> bioenergy grasses in 2008, which enabled quantification of plant-derived C accumulation owing to the natural difference in isotopic signature between C<sub>3</sub> and C<sub>4</sub> grasses (Park 1961). Soil samples were analyzed for (1) nematode abundance and diversity, (2) soil bacterial community structure and diversity, and (3) soil C and its  $\delta^{13}\text{C}$  isotopic signature.

## Methods

### Experimental Design

In 2008, a 5.4 ha experimental bioenergy agricultural field was established at the U.S. Department of Energy National Environmental Research Park at Fermilab in Batavia, IL, USA. The soil is Grays silt loam, and has a mean annual precipitation and temperature of 920 mm and 9.5°C respectively (<http://www.ncdc.noaa.gov>). In 1971, after a period of agricultural cultivation the field site was planted with non-native perennial, cool-season C<sub>3</sub> grasses (*Bromus inermis* Leyss & *Poa pratensis*) which were maintained until 1984. Biannual mowing with clippings left in place continued until

2007, at which time the standing vegetation that remained was treated with glyphosate (a broad-spectrum herbicide) and burned. Two more applications of herbicide treatment occurred in spring 2008 to prepare the area for agricultural planting of experimental plots. For more details of the planting, see Morris et al., (2016).

Six plant diversity treatments of bioenergy crops (Table 1), switchgrass and big bluestem were planted and replicated in three randomized complete blocks with fertilizer applied to half of the plant diversity treatments (0 and 67 kg N ha<sup>-1</sup>). In total, there were 36 treatment plots each 36m x 20m in size. Three switchgrass cultivars (Kanlow, Cave-In-Rock, Southlow) were planted in monoculture, and in an additional treatment were planted in polyculture to assess genotypic diversity. Plant species diversity treatments consisted of a warm season mix, with a combination of three big bluestem cultivars (Roundtree, Epic, Southlow) with the switchgrass cultivars. The most diverse plant community treatment contained three species of grasses; switchgrass and big bluestem cultivars and Indiangrass (*Sorghastrum nutans* L.), and eight species of forbs including three legumes (*Desmodium canadense*, *Lespedeza capitata*, *Dalea purpurea*). Plots were drill seeded in 20-cm rows with 6.7 kg pure live seed (PLS) at a depth of ~0.6cm with alleys of *Festuca* spp between each plot. Fertilized plots were treated with granular urea treatment (67 kg N ha<sup>-1</sup>) during the first week of June each year (Morris et al., 2016).

#### Soil Collection

In 2017 soils were sampled (2cm diameter x 15cm depth corer) from two transects established in each plot. Two transects crossed each field plot diagonally, and samples were collected 1m to the left and 1m to the right every 6m along the diagonal. This yielded a total of 12 samples across each diagonal and 24 samples per plot. The 24



individual samples collected from each plot were homogenized in the field and sent to Boise State University to be stored in -20°C freezer until processed for both biodiversity and carbon sequestration.

### Soil Processing

In the laboratory, 10g sub-samples of soil were separated through a 2mm sieve and allowed to shake overnight in 5g L<sup>-1</sup> of sodium hexametaphosphate to disperse soil particles. Shaken samples were then strained through two stacked sieves (250µm and 53µm) to separate coarse particulate organic matter (CPOM: 2mm to 250µm) and fine particulate organic matter (FPOM: 250µm to 53µm) from silt (53µm to 2µm) and clay (<2µm). Silt fractions were isolated via centrifugation in a Sorvall Legend X1R for 1 minute 22 seconds at 270 RPM. To isolate the clay fraction, 0.25 M MgCl<sub>2</sub> + CaCl<sub>2</sub> (1 mL / 100 mL) was added to remaining supernatant and the solution was further centrifuged for 10 minutes at 2000 RPM. All isolated fractions, except CPOM, were then rinsed into labeled aluminum pans with deionized water and oven dried at 65°C for 24 hours. For CPOM, sand within the samples was poured off and the remaining organic material was burned away in a muffle furnace at 400°C to determine percent of sand in each plot sample. All dried soil samples were homogenized with a ball mill prior to further analysis.

### Root Processing

Roots from each sample plot were isolated by flushing 400g of soil collected from each plot with deionized water through a 500µm sieve and rinsing roots clean. The rinsed roots were placed in labeled aluminum pans and oven dried at 70°C for 48 hours. After samples were dried, roots were stored in 6 x 9 Manila Clasp Envelopes and weighed for

root biomass. Each sample was then homogenized using a ball mill prior to further analysis.

### Mass Spectrometry

Soil and root samples were weighed into tin capsules (5x9mm, CE Elantech, Inc.) and analyzed with A 2010 ThermoFisher Delta V Plus continuous flow isotope ratio mass spectrometer which allows measurement of the stable isotopes of carbon ( $\delta^{13}\text{C}$  ‰). Glycine, cellulose and sucrose were included as standards and used to calculate %C, from which total C ( $Q_t$  in equation) was determined.

We used the following equation (Cheng, 1996; Nottingham et al., 2009) to quantify plant derived carbon input:

$$Q_p = Q_t * \frac{(\delta^{13}\text{C}_t - \delta^{13}\text{C}_s)}{(\delta^{13}\text{C}_p - \delta^{13}\text{C}_s)}$$

[Equation 1]

where  $Q_p$  is the amount of plant-derived C,  $Q_t$  is the total amount of C,  $\delta^{13}\text{C}_t$  is its isotopic composition,  $\delta^{13}\text{C}_p$  is the isotopic composition of the root material (Kanlow, Cave-in-Rock, Southlow, Switchgrass Mix = -14.15, Big Bluestem = -13.44, Prairie Mix = -13.42), and  $\delta^{13}\text{C}_s$  is the isotopic composition of the C3 soil (Bulk = -22.61, CPOM = -27.33, FPOM = -24.45, Silt = -21.70, Clay = -21.01) collected from CRP land next to the experimental field plots that was still dominated by C3 grasslands (Adkins et al., 2019).

### Bacterial Community Analysis

Bacterial community analysis was conducted by personnel at Argonne National Laboratory using procedures described in Caporaso et al., (2012; 2011). Specifically, PCR amplicon libraries targeting the 16S rRNA encoding gene present in metagenomic DNA are produced using a barcoded primer set adapted for the Illumina HiSeq2000 and

MiSeq. DNA sequence data is then generated using Illumina paired-end sequencing at the Environmental Sample Preparation and Sequencing Facility (ESPSF) at Argonne National Laboratory. Specifically, the V4 region of the 16S rRNA gene (515F-806R) is PCR amplified with region-specific primers that include sequencer adapter sequences used in the Illumina flowcell. The forward amplification primer also contains a twelve base barcode sequence that supports pooling of up to 2,167 different samples in each lane. Each 25  $\mu$ L PCR reaction contains 9.5  $\mu$ L of MO BIO PCR Water (Certified DNA-Free), 12.5  $\mu$ L of QuantaBio's AccuStart II PCR ToughMix (2x concentration, 1x final), 1  $\mu$ L Golay barcode tagged Forward Primer (5  $\mu$ M concentration, 200 pM final), 1  $\mu$ L Reverse Primer (5  $\mu$ M concentration, 200 pM final), and 1  $\mu$ L of template DNA. The conditions for PCR are as follows: 94°C for 3 minutes to denature the DNA, with 35 cycles at 94°C for 45 s, 50°C for 60s, and 72°C for 90 s; with a final extension of 10 min at 72°C to ensure complete amplification. Amplicons are then quantified using PicoGreen (Invitrogen) and a plate reader (Infinite® 200 PRO, Tecan). Once quantified, volumes of each of the products are pooled into a single tube so that each amplicon is represented in equimolar amounts. This pool is then cleaned up using AMPure XP Beads (Beckman Coulter), and then quantified using a fluorometer (Qubit, Invitrogen). After quantification, the molarity of the pool is determined and diluted down to 2nM, denatured, and then diluted to a final concentration of 6.75 pM with a 10% PhiX spike for sequencing on the Illumina MiSeq. Amplicons are sequenced on a 151bp x 12bp x 151bp Micro MiSeq run using customized sequencing primers and procedures.

Sequencing data was analyzed using the pipeline software package QIIME1.9. Paired-end reads were joined without trimming, but singletons were removed. OTU'S

were clustered by 97% similarity using the Greengenes database to assign taxonomy to species. The phylogenetic tree created has 192298 tips and 192296 internal nodes. There were no negative controls or blanks sequenced and no contaminant OTU sequences were removed.

### Nematode Analysis

Nematodes were extracted using the sugar flotation method based on (Jenkins, 1964). Soil samples were suspended in tap water and rinsed through stacked sieves (180 $\mu$ m and 38 $\mu$ m). Soil material in the 38 $\mu$ m sieve was rinsed into 50mL round-bottomed centrifuge tubes using tap water and were then centrifuged for 10 minutes at 3000 rpm. Supernatant was discarded and a sucrose sugar solution (454g L<sup>-1</sup>) was then added to the samples. Working quickly to not expose the nematodes to osmotic stress, this solution was centrifuged for 2 minutes at 3000 rpm. The sugar supernatant was poured through a 25 $\mu$ m sieve and contents in the sieve were rinsed into 50mL tubes and stored at 4°C until further analysis. To fix nematodes for morphological identification, liquid was first aspirated from the tubes. Then, 90°C 4% formaldehyde was added to the tubes (which contained nematodes) followed immediately by cold a similar amount of 4% formaldehyde. Nematodes were then counted and identified according to mouthparts and feeding group (Yeates et al., 1993).

### Statistical Analysis

All ANOVA tests for treatment effects on soil C and soil biodiversity were performed in R (package Car; Fox & Weisberg, 2019) and JMP (JMP®, Version <15>. SAS Institute Inc., Cary, NC, 1989-2019) and post-hoc analysis was performed using Tukey HSD. Alpha ( $\alpha$ -) diversity metrics, which measure average species diversity in a

local habitat, included Simpson and Shannon indexes. Bacterial beta ( $\beta$ -) diversity, the ratio between  $\alpha$ - and regional diversity, was calculated as weighted UniFrac and analyzed using PERMANOVA in R (package Vegan, Oksanen et al., 2019) to create non-metric multidimensional scaling (NMDS) plots. After obtaining PERMANOVA results, replicates (n=3) were pooled together based on diversity treatments and presence or absences of N fertilizer. Statistical significance was based on an alpha value of  $p < 0.05$ .

## Results

### Soil Community Structure and Biodiversity

Plant community diversity or fertilizer application did not affect  $\alpha$ -diversity of nematodes, regardless of feeding group (*Table 2*). There were no significant interactions between plant diversity and fertilizer treatment. However, nematode family composition was significantly impacted by fertilizer application (*Figure 1*). Specifically, we found that the relative abundance of Criconematidae (plant feeders) decreased in response to N addition, whereas the relative abundance of Cephalobidae (bacterial feeders) increased in response to N addition (*Figure 2*).

Bacterial  $\alpha$ -diversity was not affected by plant species diversity or by fertilizer application (*Table 2*). However, bacterial  $\beta$ -diversity decreased significantly with N application (*Figure 3a*). In addition, the bacterial community composition (OTU relative abundance) changed significantly in response to N fertilizer application (*Figure 3b*). We found that the OTU abundance of the bacterial phyla Crenarchaeota and Chlamydiae increased in response to N addition, while the OTU abundance of Acidobacteria, Chloroflexi, GAL15, WS2 and Planctomycetes decreased in response to N addition (*Figure 4, Table 3*).

Both soil nematode and bacterial community structures varied significantly among monocultures of the switchgrass cultivars. The relative abundance of the nematode superfamily Dorylaimoidea was higher in soils planted with Cave-in-Rock than soils planted with Kanlow (*Figure 6*). For bacteria, the relative abundances of Acidobacteria, Nitrospirae, OP3, Planctomycetes and WS3 were higher in soils planted with Cave-in-Rock than in soils planted with Southlow and Kanlow, and the relative abundances of Bacteroidetes, Chlorobi, Cyanobacteria, Fibrobacteres, GN02 were higher in soils planted with Kanlow than in soils planted with Southlow and Cave-in-Rock. Finally, Chlamydiae, OD1, OP11, TM6 and TM7 had higher relative abundances in soils planted with Southlow than in soils planted with Kanlow and Cave-in-Rock plots (*Figure 5*).

#### Soil C Sequestration

Neither plant species diversity nor N fertilizer application affected root biomass production (*Table 4*). Similarly, neither of those treatments had a significant effect on total soil C concentrations either in bulk soil, or in the four soil fractions (*Table 4*). N fertilizer significantly increased total N in the CPOM fraction and the interaction of N fertilizer and plant composition was significant in the silt fraction (*Table 4*).

Plant-derived C in bulk soils or fractions was also not significantly affected by N fertilization (*Table 5*). However, there was a significant effect of plant diversity treatment on plant derived C in the bulk soil (*Table 5*) and CPOM (*Table 5, Figure 7, Table 6*). Specifically in the latter case, there was significantly more plant derived C in bulk soil of plots planted with big bluestem than in the other plant diversity treatments (*Figure 7*). Likewise, the  $\delta^{13}\text{C}$  signature of CPOM-C in the big bluestem plots was significantly less

negative than the  $\delta^{13}\text{C}$  signature of CPOM-C in the Prairie Mix plots (  $-14.28\text{‰}$  vs.  $-18.45\text{‰}$ , respectively) (*Table 5*), indicating a greater input of plant-derived C in soils planted with the big bluestem mix, than in other species mixes (*Table 5*). Plant diversity also significantly impacted the  $\delta^{13}\text{C}$  of each soil fraction (*Table 5*). Plant-derived C was also significantly impacted by switchgrass cultivar. We found less plant derived C in soils dominated by Kanlow than in soils dominated by Cave-in-Rock and Southlow (*Table 5*).

### **Discussion**

This study yielded three main results: [1] both fertilizer application and switchgrass cultivar, but not aboveground plant community diversity, influenced the soil nematode family composition, [2] fertilizer addition significantly reduced bacterial  $\beta$ -diversity and both fertilizer application and switchgrass cultivar affected the bacterial community composition, [3] big bluestem impacted soil C storage, but plant diversity and N fertilizer did not. In summary, our data indicate that choice of plant species or cultivar has a greater impact on soil C sequestration than increasing aboveground plant diversity or applying N fertilizer. While N fertilization did not affect soil C sequestration, it negatively affected bacterial diversity, and altered the community structure of nematodes and bacteria. Further study is warranted given that these changes may have contributed to the lack of a positive effect of N fertilization on soil C accumulation. If alteration of soil bacteria and nematodes influences the efficacy of fertilization on soil C accumulation, this effect would have to be considered when managing bioenergy cropping systems.

#### **Soil Community Structure and Biodiversity**

Neither inter- nor intra specific plant diversity significantly altered the community structure or biodiversity of soil nematodes. This result is not in agreement with previous

research in other ecosystems where an increase in plant species diversity or plant functional group diversity increased taxon richness of plant-feeding, bacterial-feeding and predatory nematodes (Cortois et al, 2017). Increased aboveground plant community composition might stimulate biodiversity of soil fauna by creating a more heterogeneous microenvironment both spatially and temporally (Eisenhauer, 2016), or by increasing the quantity and chemical diversity of plant-derived C substrates into soil. For example, greater plant species diversity has been shown to increase resource availability for plant feeding nematodes (Sohlenius et al., 2011), and bacterial-feeding nematodes through the impact on the soil bacterial community composition (Wardle et al., 2006; Eisenhauer et al., 2010; De Deyn et al., 2004). Previous research has shown that root exudates vary among cultivars of switchgrass and big bluestem when planted in monoculture (Kelly-Slatten et al., 2019), and in the present study we found that cultivars differentially affected the soil bacterial composition, suggesting that different cultivars can modulate physical, chemical and biological soil properties in a manner that affects soil nematodes. Given this result, we might expect an increase in the diversity of substrates available to the soil nematode community when we mix species and cultivars in our system. However, increasing intra-or inter-specific diversity did not affect soil nematode family composition or diversity. Such effects may have been apparent if we had species-specific data on soil nematodes, rather than analyses done at the level of family. Further study is needed to assess this possibility.

Inter, or intra specific plant diversity also did not significantly impact the community structure or biodiversity of soil bacteria, though the bacterial community composition differed significantly among monocultures of switchgrass cultivars. Revillini



et al. (2019) analyzed impacts of aboveground biodiversity on soil bacterial diversity at the same field site after seven growing seasons. They also found  $\alpha$ -diversity of the soil bacterial community was not affected by aboveground biodiversity and that there were cultivar specific impacts on the soil bacterial community structure. These cultivar specific impacts on soil bacterial communities are likely grounded in differences in root-derived C inputs either through exudates, or root litter, which drive differences in rhizosphere chemistry among cultivars (Kelly-Slatten et al., 2019; Stewart et al., 2017). The lack of a relationship between aboveground plant community diversity and  $\alpha$ -diversity of the soil bacterial community has been observed by others (Kowalchuk et al., 2002; Fierer et al., 2007; Prober et al., 2015). It has been proposed that there is an uncoupling of above- and belowground biodiversity (Wardle et al., 2006), and that belowground biodiversity is likely driven to a greater extent by edaphic factors, such as soil pH (Fierer et al., 2007; Tedersoo et al., 2014). In addition, the immense heterogeneity of the soil organismal community, and particularly the bacterial community may explain why we were unable to detect an effect of plant community on soil organismal diversity.

Nitrogen application significantly altered the soil nematode community composition, and the direction of the response to N addition varied among nematode feeding groups, as seen in other studies (Sarathchandra et al., 2001; Liang et al., 2009). We found an increase in the abundance of bacteria feeding nematodes, particularly in the Cephalobidae family following N addition. This may be driven by an increase in the abundance and biomass of the soil bacterial community owing to fertilizer induced increases in  $\text{NO}_3^-$  concentrations (Song et al., 2016; Wei et al., 2012; Liu et al., 2016), or root exudate inputs (Badri and Vivanco, 2009). The effects of N fertilization on plant

feeding nematodes were variable, which is common (Liang et al., 2009) because N addition can change patterns of belowground C allocation (Sun et al., 2013). Plant feeding nematodes are strongly dependent on the effects of fertilizer inputs on specific plant species and is as-such driven by seasonality of plant productivity, soil temperature and moisture availability (Verschoor et al., 2001). While N fertilization altered the nematode community composition in our study, it did not affect diversity of the soil nematode community. A recent meta-analysis indicated that soil faunal diversity is generally not negatively impacted by N fertilization, unless N fertilizer is applied in excess of 150 kg ha<sup>-1</sup> (de Graaff et al., 2019). Our soils received 67 kg N ha<sup>-1</sup> which may explain the lack of an impact on nematode diversity. Our study indicates that the soil nematode community is sensitive to N fertilization, as Wei et al., (2012) found, and this sensitivity can lead to significant shifts in microbial community composition that may feedback to affect ecosystem functioning.

Fertilizer use significantly reduced bacterial  $\beta$ -diversity, corroborating the results of other studies (Choudhary et al., 2018; Shen et al., 2010; Yevdokimov et al., 2008; Zeng et al., 2016). Our plots were fertilized with granular urea, which can increase ionic strength and reduce soil pH thus inhibiting some soil microorganisms and favoring others (Eno et al., 1955; Omar and Ismail, 1999; Magdoff et al., 1997; Fierer and Jackson, 2006; Zhang et al., 2014). The positive effect of N fertilizer on bacterial diversity may be a consequence of fertilizer-induced increases in soil C (Belay-Tedla et al., 2009; Zhang et al., 2017; Chen et al., 2018; Rasse et al., 2005), which enhance resource availability to soil microbes (Hao and Chang, 2002; Mooleki et al., 2002), buffer against fluctuations in pH, and improve soil structure (Miller et al., 2002; Whalen and Chang, 2002; Reynolds et

al., 2003). However, in our study we removed plant biomass from the plots on a yearly basis and N fertilization did not impact soil C, which may explain why bacterial diversity in our experiment was reduced even at an application rate of 67 kg N ha<sup>-1</sup>. In addition to the loss in bacterial diversity, the relative abundance of Acidobacteria and Chloroflexi decreased with N fertilizer application. This result may be explained by the oligotrophic life history of these organisms, which leads to slower growth rates and lower competitive ability at higher resource availability (Fierer et al., 2012). In contrast, the relative abundance of Crenarchaeota and Chlamydiae increased in response to N fertilization. This result corroborates Revillini et al. (2019) and is likely explained by the role of these bacteria in ammonia oxidation (Weidler et al., 2008; Xiao et al., 2010). Our study indicates that low levels of sustained synthetic N inputs (i.e. 67 kg N ha<sup>-1</sup>) can significantly impact the diversity and structure of soil microorganisms and perhaps ecosystem functioning.

#### Soil C Sequestration

N fertilizer did not impact soil C accumulation, as other studies in cellulosic bioenergy cropping systems have found (Das et al., 2018; Kibet et al., 2015; Jungers et al., 2017; Valdez et al. 2017). Fertilization can lead to increased decomposition of soil organic carbon (Khan et al., 2007), especially if N fertilizer application enhances the input of C that is easily accessible to the soil microbial community (Lin et al., 2019). Our results indicate that the N concentration in the CPOM fraction was greater in N fertilized soils, indicating that N fertilization enhances litter quality and possibly decomposition rates at our site. We also found that N fertilizer application significantly increased aboveground plant biomass production across all treatments (as sampled by Morris et al.,

2016), which may have increased labile soil C inputs through greater litter inputs. Alternatively, N fertilizer might have not affected soil C accumulation in our experiment, because it did not impact switchgrass root production, which has been found in other studies (Kibet et al., 2015), and root-derived C inputs which are key to soil C accumulation (Rasse et al., 2005). Finally, the lack of a response to N fertilizer inputs have been the result of an increase in the relative abundance of bacteria feeding nematodes, which can decrease microbial biomass (Djigal et al., 2010; Trap et al., 2016). Microbial biomass formation is an important precursor to soil C stabilization (Kallenbach et al., 2015; 2016), thus a change in soil microbial biomass can reduce soil C accumulation (Emery et al., 2017; Lupwayi et al., 2005). While it is unclear which mechanisms best explain why N fertilization did not promote soil C accumulation at our site, our study highlights that understanding belowground dynamics at the root-soil organism interface and their response to N fertilizer inputs is crucial to predicting soil C sequestration in bioenergy cropping systems.

We found no effect of inter,- or intra-specific plant community diversity on soil C sequestration, in contrast to previous research (Chen et al., 2018; Díaz et al., 2009). Positive impacts of aboveground plant community diversity on soil C accumulation may be explained by an increased variation in plant traits, such as chemical composition of root exudates that influence the composition of soil heterotrophs and their impact on soil C accrual (De Deyn et al., 2008; Bezemer et al., 2006). Others have found that increasing plant diversity promotes microbial growth and biomass production, which yields greater microbial necromass that is preferentially stabilized in soil (Liu et al., 2007; Chen et al., 2018; Prommer et al., 2019). We contend that aboveground plant community diversity at

our site did not impact microbial biomass or soil C accumulation because the annual removal of plant biomass may have significantly reduced soil C inputs (Steinbeiss et al., 2007). This effect may have been compounded by a lack of increase in root biomass with increasing plant diversity (Carney and Matson, 2005). While we found no links between above- and belowground biodiversity or soil C sequestration, plant community diversity will likely positively impact ecosystem structure and functioning in ways we did not measure, and as such should not be discounted as a successful management strategy.

We found strong cultivar and species-specific effects on soil C accumulation. In particular, big bluestem contributed significantly more C to the soil C pool than other plant species (see also Adkins et al., 2019), unlike other studies that reported no differences in soil C sequestration between big bluestem and other perennial grasses (Kibet et al., 2015; Evers et al., 2013). Greater plant-derived C in soils planted with big bluestem at our site may be related to greater litter input in these systems. Indeed, plots containing big bluestem produced more biomass (Morris et al., 2016), and we found greater retention of plant derived C in the CPOM fraction in soils planted with big bluestem. The greater association of C associated with the less persistent CPOM fraction suggests that big bluestem derived C may not be protected in the long-term (Jastrow et al., 2018). Alternatively, increased particulate organic matter (POM) inputs may enhance microbial biomass and ultimately C stabilization on protected soil surfaces (Helal and Sauerbeck, 1986). We also found that the cultivars Southlow and Cave-in-Rock accrued more soil C than the cultivar Kanlow, which may be driven by differences in soil C input owing to variability in root morphology (Adkins et al., 2016), or by differences in retention of root derived C (Kelly-Slatten et al., 2019). Understanding the relationships

between the quantity and chemical composition of root-derived input, and the consequences for soil microbial metabolism will improve our understanding of soil C accumulation under different cultivars of switchgrass.

### **Conclusion**

This study highlights that management decisions in cellulosic bioenergy cropping systems, including selection of plant species and cultivar, and N fertilization rates can significantly impact belowground biodiversity and community composition of soil bacteria and soil fauna, as well as soil C accumulation. In contrast to our hypotheses N fertilizer inputs did not increase soil C accumulation, but it did reduce belowground diversity. Conversely, plant species and cultivar were strong predictors of soil C accumulation. While aboveground plant community diversity did not affect belowground biodiversity or soil C accumulation, it is likely to positively impact other ecosystem attributes and processes that were not measured in this study. For example, Morris et al. (2016) showed that increasing intraspecific biodiversity in switchgrass dominated bioenergy cropping systems can stabilize yields, and others have shown a multitude of benefits associated with increasing plant community diversity in agroecosystems that reach across trophic levels (Lange et al., 2015; Hooper et al., 2012; Norris, 2008). This along with other studies that explore the implications of land-use change for bioenergy production will ensure that we sustainably manage land for biomass production and soil C sequestration and soil organismal biodiversity.

## Tables

**Table 1** Description of plant treatments in the agronomic trial. Each plant treatment received a split-plot fertilization regimen.

Plant Diversity Treatment	Species description
<b>Switchgrass Monocultures</b> (1 species, 1 cultivar)	Kanlow, Cave-in-Rock, and Southlow switchgrass cultivars planted in monoculture
<b>Switchgrass Mix (SG)</b> (1 species, 3 cultivars)	Kanlow, Cave-in-Rock, and Southlow switchgrass cultivars planted in polyculture
<b>Big Bluestem Mix (BB)</b> (2 species, 6 cultivars)	Three switchgrass cultivars planted with three big bluestem cultivars (Roundtree, Epic, and Southlow)
<b>Prairie Mix (PR)</b> (12 species, 6 cultivars)	Eleven species polyculture consisting of three grass species and eight forb species, three of which are legumes. Grasses: the three switchgrass cultivars, the three big bluestem cultivars, Indiangrass. Forbs: showy tick trefoil, round-headed bush clover, purple prairie clover, tall tickseed, smooth oxeye, yellow coneflower, New England aster, Culver's root.

**Table 2**      **Alpha diversity metrics for soil organisms. (means  $\pm$  SE; n=3).**

<b>Treatment</b>		<b>16s Bacterial DNA</b>	<b>Nematode</b>	
		Shannon Diversity	Shannon Diversity	Simpson Diversity
<b>KA</b>	<i>Fertilized</i>	10.07 $\pm$ 0.21	1.69 $\pm$ 0.16	0.95 $\pm$ 0.015
	<i>Unfertilized</i>	9.62 $\pm$ 0.12	1.72 $\pm$ 0.15	0.98 $\pm$ 0.009
<b>CR</b>	<i>Fertilized</i>	9.25 $\pm$ 0.14	1.72 $\pm$ 0.08	0.96 $\pm$ 0.007
	<i>Unfertilized</i>	9.14 $\pm$ 0.34	2.19 $\pm$ 0.14	0.98 $\pm$ 0.003
<b>SL</b>	<i>Fertilized</i>	9.93 $\pm$ 0.30	1.75 $\pm$ 0.12	0.97 $\pm$ 0.006
	<i>Unfertilized</i>	9.46 $\pm$ 0.14	1.92 $\pm$ 0.18	0.97 $\pm$ 0.002
<b>SG</b>	<i>Fertilized</i>	9.52 $\pm$ 0.18	1.90 $\pm$ 0.14	0.97 $\pm$ 0.001
	<i>Unfertilized</i>	9.06 $\pm$ 0.39	1.88 $\pm$ 0.04	0.97 $\pm$ 0.004
<b>BB</b>	<i>Fertilized</i>	9.57 $\pm$ 0.30	1.78 $\pm$ 0.10	0.98 $\pm$ 0.003
	<i>Unfertilized</i>	9.80 $\pm$ 0.50	1.73 $\pm$ 0.15	0.98 $\pm$ 0.002
<b>PR</b>	<i>Fertilized</i>	9.38 $\pm$ 0.27	1.93 $\pm$ 0.15	0.98 $\pm$ 0.006
	<i>Unfertilized</i>	9.49 $\pm$ 0.36	1.90 $\pm$ 0.11	0.94 $\pm$ 0.005
<b>Source of ANOVA Variation (p-values)</b>				
Plant Diversity		0.218	0.403	0.322
N Fertilizer		0.266	0.224	0.649
Diversity $\times$ N Fert		0.723	0.359	0.061



**Table 3      2-Way Analysis of variation associated with the means of values represented in Figure 4 (n=3).**

Source of Variation ANOVA	<i>N Fertilizer</i>		
	Fertilized Mean	Unfertilized Mean	p-value
Crenarchaeota	1.526	0.838	<b>0.023</b>
Acidobacteria	12.915	14.743	<b>0.002</b>
Chlamydiae	0.367	0.258	<b>0.031</b>
Chloroflexi	2.933	4.290	<b>0.033</b>
GAL15	0.001	0.002	<b>0.042</b>
Planctomycetes	6.169	7.103	<b>0.044</b>
WS2	0.001	0.002	<b>0.002</b>

**Table 4**      **Root biomass, Total C & Total N for experimental treatments (means  $\pm$  SE, n=3).**

Plant Diversity	N Fertilizer (kg ha <sup>-1</sup> )	Root Biomass (g/ 400g soil)	Total C (mg C/ g soil)					Total N (mg N/ g soil)				
			<i>BULK</i>	<i>CPOM</i>	<i>FPOM</i>	<i>SILT</i>	<i>CLAY</i>	<i>BULK</i>	<i>CPOM</i>	<i>FPOM</i>	<i>SILT</i>	<i>CLAY</i>
Kanlow	0	5.35 $\pm$ 1.32	24.01 $\pm$ 2.26	423.48 $\pm$ 16.43	38.47 $\pm$ 4.71	13.83 $\pm$ 1.62	45.18 $\pm$ 4.03	2.06 $\pm$ 0.20	5.45 $\pm$ 1.48	2.95 $\pm$ 0.34	1.45 $\pm$ 0.17	5.13 $\pm$ 0.36
	67	3.65 $\pm$ 0.59	26.64 $\pm$ 2.17	382.51 $\pm$ 50.07	35.65 $\pm$ 6.77	10.85 $\pm$ 0.66	40.58 $\pm$ 7.16	2.35 $\pm$ 0.14	6.83 $\pm$ 0.05	2.82 $\pm$ 0.51	1.07 $\pm$ 0.12	4.37 $\pm$ 0.62
Cave-in-Rock	0	5.13 $\pm$ 0.39	25.76 $\pm$ 1.99	431.31 $\pm$ 8.25	33.90 $\pm$ 1.31	14.42 $\pm$ 1.33	45.33 $\pm$ 3.85	2.24 $\pm$ 0.18	3.95 $\pm$ 0.98	2.55 $\pm$ 0.32	1.60 $\pm$ 0.07	4.74 $\pm$ 0.39
	67	5.43 $\pm$ 1.08	24.45 $\pm$ 2.28	470.95 $\pm$ 66.32	42.04 $\pm$ 10.09	11.12 $\pm$ 0.12	38.81 $\pm$ 2.04	2.17 $\pm$ 0.18	7.33 $\pm$ 0.39	3.36 $\pm$ 0.88	1.19 $\pm$ 0.02	4.02 $\pm$ 0.37
Southlow	0	3.84 $\pm$ 0.45	31.46 $\pm$ 2.06	401.67 $\pm$ 10.28	169.13 $\pm$ 16.43	12.79 $\pm$ 0.80	48.15 $\pm$ 5.34	2.55 $\pm$ 0.14	5.21 $\pm$ 0.34	3.12 $\pm$ 0.83	1.28 $\pm$ 0.11	5.31 $\pm$ 0.51
	67	5.08 $\pm$ 0.57	12.25 $\pm$ 1.42	399.74 $\pm$ 14.42	63.66 $\pm$ 2.37	13.37 $\pm$ 0.35	37.25 $\pm$ 1.92	2.02 $\pm$ 0.17	8.18 $\pm$ 0.51	4.59 $\pm$ 0.33	1.36 $\pm$ 0.08	4.34 $\pm$ 0.58
Switchgrass Mix	0	4.65 $\pm$ 0.46	22.84 $\pm$ 1.66	405.40 $\pm$ 30.49	59.40 $\pm$ 4.91	13.58 $\pm$ 0.26	46.15 $\pm$ 6.94	1.99 $\pm$ 0.14	5.36 $\pm$ 0.34	4.14 $\pm$ 0.30	1.49 $\pm$ 0.03	4.35 $\pm$ 0.60
	67	3.54 $\pm$ 0.52	8.31 $\pm$ 2.42	413.52 $\pm$ 20.58	53.09 $\pm$ 5.26	11.11 $\pm$ 1.58	35.45 $\pm$ 7.81	2.11 $\pm$ 0.34	4.87 $\pm$ 1.02	4.04 $\pm$ 0.22	1.18 $\pm$ 0.20	4.42 $\pm$ 0.67
Big Bluestem Mix	0	7.64 $\pm$ 0.54	28.21 $\pm$ 3.27	364.42 $\pm$ 71.28	35.61 $\pm$ 1.64	12.18 $\pm$ 0.62	39.65 $\pm$ 2.34	2.29 $\pm$ 0.21	3.56 $\pm$ 0.85	2.71 $\pm$ 0.23	1.13 $\pm$ 0.08	4.07 $\pm$ 0.39
	67	4.97 $\pm$ 0.99	27.84 $\pm$ 1.17	435.81 $\pm$ 15.90	37.94 $\pm$ 3.42	16.17 $\pm$ 1.33	39.39 $\pm$ 5.64	2.35 $\pm$ 0.06	5.16 $\pm$ 0.43	3.07 $\pm$ 0.34	1.67 $\pm$ 0.18	4.81 $\pm$ 0.24
Prairie Mix	0	3.61 $\pm$ 1.27	25.14 $\pm$ 1.16	360.08 $\pm$ 65.49	49.55 $\pm$ 0.06	11.94 $\pm$ 0.40	41.27 $\pm$ 4.97	2.09 $\pm$ 0.08	5.24 $\pm$ 0.57	3.93 $\pm$ 1.13	1.39 $\pm$ 0.18	4.26 $\pm$ 0.72
	67	3.87 $\pm$ 1.04	29.77 $\pm$ 3.50	437.26 $\pm$ 8.37	63.60 $\pm$ 17.91	21.59 $\pm$ 2.81	45.82 $\pm$ 9.00	2.51 $\pm$ 0.28	5.18 $\pm$ 1.33	4.93 $\pm$ 1.44	2.17 $\pm$ 0.32	4.90 $\pm$ 0.99

Source of variation (ANOVA)

Plant Diversity	0.062	0.143	0.699	0.762	0.779	0.794	0.724	0.123	0.114	<b>0.033</b>	0.957
N Fertilizer	0.215	0.940	0.232	0.779	0.833	0.083	0.520	<b>0.002</b>	0.167	0.568	0.710
Diversity $\times$ N Fert	0.218	0.117	0.628	0.623	0.691	0.879	0.248	0.271	0.826	<b>0.002</b>	0.432

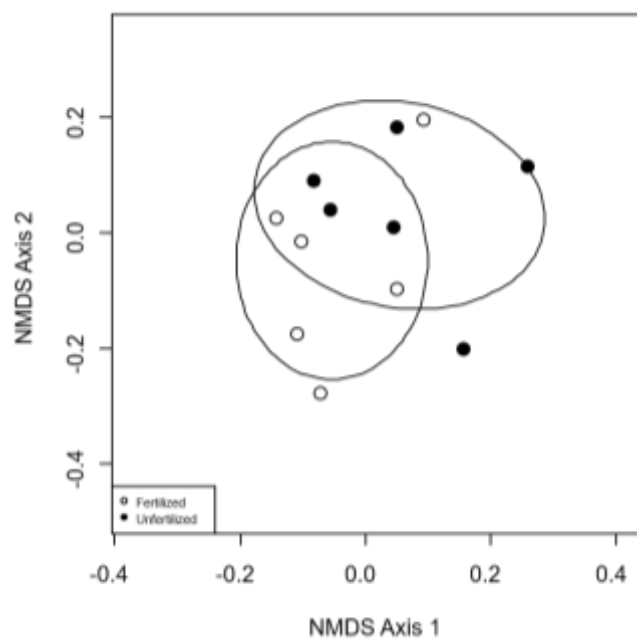
**Table 5**  $\delta^{13}\text{C}$  and Plant-derived ( $\text{C}_4$ ) values for all treatment plots (means  $\pm$  SE, n=3).

Plant Diversity	N Fertilizer (kg ha <sup>-1</sup> )	$\delta^{13}\text{C}$ (‰)					Plant Derived ( $\text{C}_4$ ) C (mg/ g soil)				
		<i>BULK</i>	<i>CPOM</i>	<i>FPOM</i>	<i>SILT</i>	<i>CLAY</i>	<i>BULK</i>	<i>CPOM</i>	<i>FPOM</i>	<i>SILT</i>	<i>CLAY</i>
Kanlow	0	-21.56 $\pm$ 0.34	-21.15 $\pm$ 0.76	-23.30 $\pm$ 0.36	-21.76 $\pm$ 0.29	-19.51 $\pm$ 0.22	2.81 $\pm$ 0.77	205.26 $\pm$ 24.54	4.06 $\pm$ 0.84	0.23 $\pm$ 0.23	9.84 $\pm$ 1.70
	67	-21.85 $\pm$ 0.37	-19.71 $\pm$ 0.38	-22.94 $\pm$ 0.61	-21.34 $\pm$ 0.49	-19.15 $\pm$ 0.42	2.60 $\pm$ 1.40	230.29 $\pm$ 41.60	9.64 $\pm$ 6.05	0.83 $\pm$ 0.51	11.99 $\pm$ 3.59
Cave-in-Rock	0	-19.94 $\pm$ 0.20	-17.05 $\pm$ 0.78	-21.22 $\pm$ 0.56	-20.67 $\pm$ 0.13	-18.73 $\pm$ 0.25	8.12 $\pm$ 0.90	349.25 $\pm$ 35.34	10.71 $\pm$ 2.01	2.00 $\pm$ 0.43	15.34 $\pm$ 2.94
	67	-20.74 $\pm$ 0.50	-17.50 $\pm$ 0.69	-20.99 $\pm$ 0.17	-20.94 $\pm$ 0.26	-19.24 $\pm$ 0.44	5.64 $\pm$ 1.10	357.86 $\pm$ 75.35	13.87 $\pm$ 2.76	4.05 $\pm$ 3.33	10.26 $\pm$ 3.04
Southlow	0	-20.78 $\pm$ 0.13	-17.90 $\pm$ 0.41	-22.89 $\pm$ 0.52	-20.62 $\pm$ 0.43	-18.79 $\pm$ 0.48	7.61 $\pm$ 0.93	287.99 $\pm$ 19.72	5.62 $\pm$ 1.29	1.96 $\pm$ 0.66	15.47 $\pm$ 3.79
	67	-20.67 $\pm$ 0.51	-18.85 $\pm$ 0.17	-21.48 $\pm$ 0.11	-22.10 $\pm$ 0.35	-19.60 $\pm$ 0.22	5.39 $\pm$ 0.60	256.90 $\pm$ 5.64	18.30 $\pm$ 0.32	0.14 $\pm$ 0.14	9.31 $\pm$ 2.09
Switchgrass Mix	0	-20.31 $\pm$ 0.33	-17.93 $\pm$ 0.67	-21.41 $\pm$ 0.39	-21.54 $\pm$ 0.07	-19.50 $\pm$ 0.51	6.09 $\pm$ 0.52	291.08 $\pm$ 37.94	17.12 $\pm$ 3.25	0.29 $\pm$ 0.12	10.81 $\pm$ 4.06
	67	-19.92 $\pm$ 0.56	-16.23 $\pm$ 0.60	-20.83 $\pm$ 0.17	-20.86 $\pm$ 0.34	-18.96 $\pm$ 0.58	7.13 $\pm$ 0.24	364.41 $\pm$ 5.48	18.82 $\pm$ 2.68	1.68 $\pm$ 0.60	14.37 $\pm$ 12.61
Big Bluestem Mix	0	-19.39 $\pm$ 0.13	-14.28 $\pm$ 0.17	-19.97 $\pm$ 0.15	-20.37 $\pm$ 0.37	-18.60 $\pm$ 0.34	9.83 $\pm$ 0.80	397.56 $\pm$ 36.79	15.00 $\pm$ 0.67	1.96 $\pm$ 0.54	12.75 $\pm$ 2.25
	67	-19.60 $\pm$ 0.18	-15.57 $\pm$ 0.76	-19.85 $\pm$ 0.58	-20.54 $\pm$ 0.38	-18.59 $\pm$ 0.32	9.06 $\pm$ 0.91	370.84 $\pm$ 36.49	16.20 $\pm$ 3.26	2.28 $\pm$ 0.85	17.33 $\pm$ 3.68
Prairie Mix	0	-20.71 $\pm$ 0.23	-18.45 $\pm$ 0.73	-21.81 $\pm$ 0.46	-21.87 $\pm$ 0.24	-19.61 $\pm$ 0.27	5.14 $\pm$ 0.48	236.07 $\pm$ 38.75	16.82 $\pm$ 4.34	0.11 $\pm$ 0.08	7.32 $\pm$ 0.99
	67	-20.82 $\pm$ 0.15	-17.82 $\pm$ 0.73	-21.95 $\pm$ 0.36	-21.69 $\pm$ 0.51	-19.30 $\pm$ 0.81	6.15 $\pm$ 0.42	317.44 $\pm$ 25.03	15.36 $\pm$ 5.29	0.94 $\pm$ 0.94	11.69 $\pm$ 5.71
<b>Source of variation (ANOVA)</b>											
Plant Community		0.428	<0.001	<0.001	0.003	0.490	<0.001	0.002	0.495	0.834	0.595
Nitrogen Fertilizer		0.360	0.435	0.056	0.655	0.702	0.177	0.159	0.725	0.304	0.086
Community $\times$ N Fert		0.819	0.196	0.762	0.031	0.535	0.128	0.557	0.525	0.713	0.758

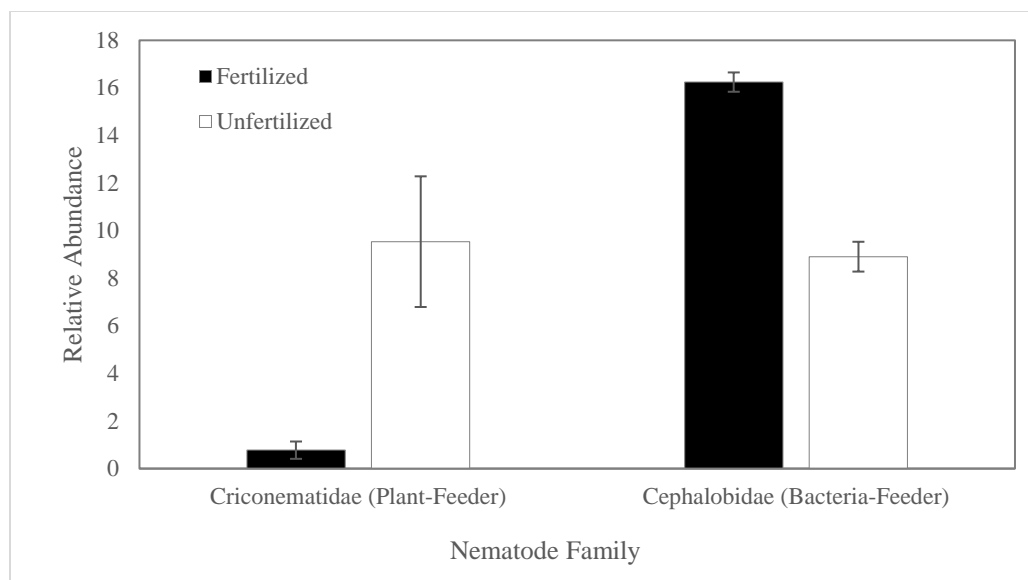
**Table 6          2-Way Analysis of variation p-values associated with the means of values represented in Figure 7- “Plant (C<sub>4</sub>) derived C content normalized to the weight of soil fraction (%) in 1 g bulk soil (bars are means  $\pm$  SE, n=6).”**

Source of Variation ANOVA			
	N Fertilizer	Plant Diversity	Diversity $\times$ N Fert
BULK	0.612	<b>0.006</b>	0.313
CPOM	0.159	<b>0.002</b>	0.557
FPOM	0.725	0.495	0.525
SILT	0.304	0.834	0.713
CLAY	0.086	0.595	0.758

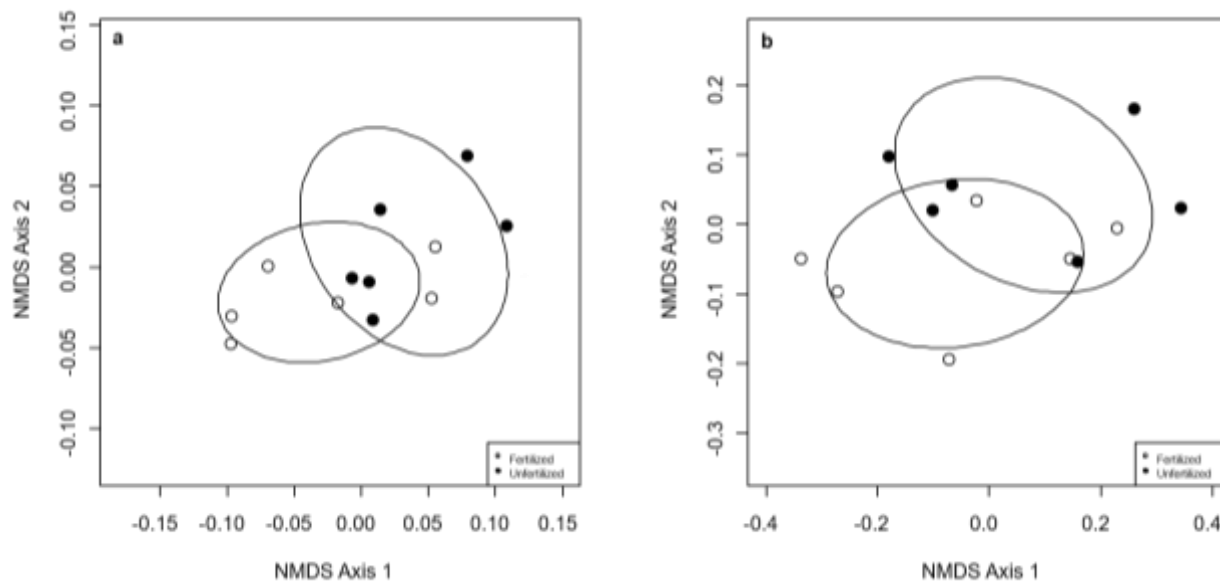
## Figures



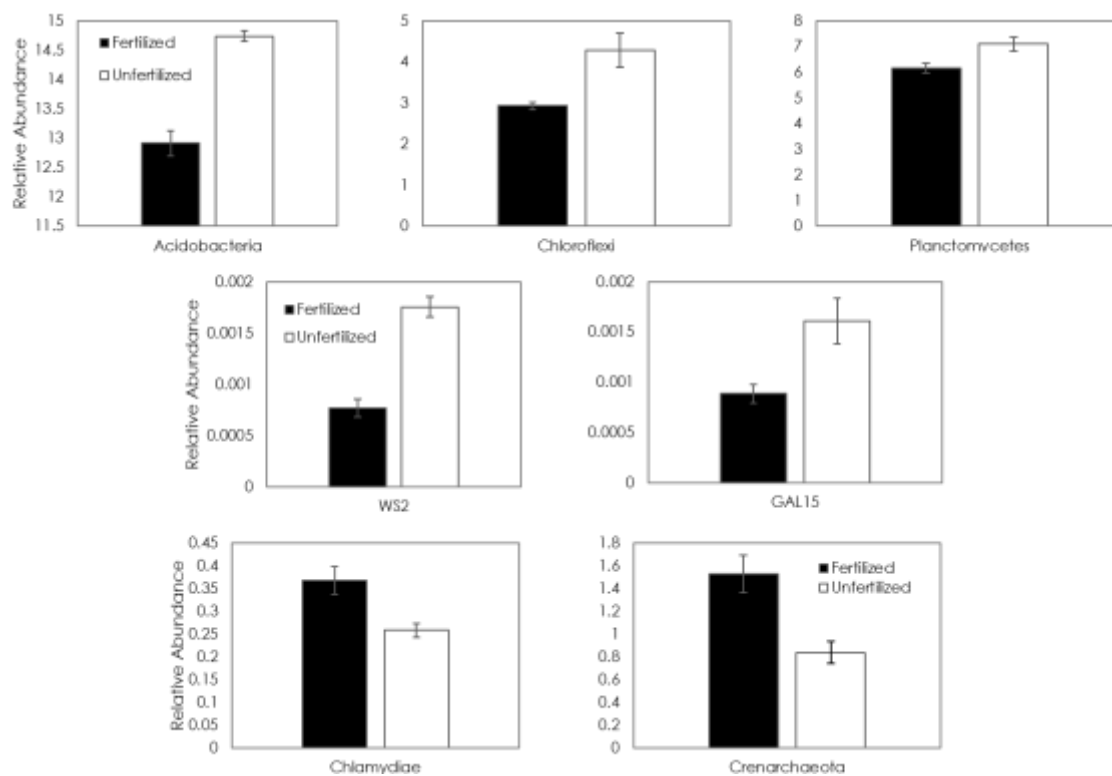
**Figure 1** Non-metric multidimensional scaling (NMDS) of nematode family relative abundance (ellipses are standard deviation, stress: 0.2543563, n=6).



**Figure 2** Relative abundance of 2 nematode families (bars are means  $\pm$  SE,  $n=3$ ) within 2 defined feeding groups as a function of fertilizer treatment.

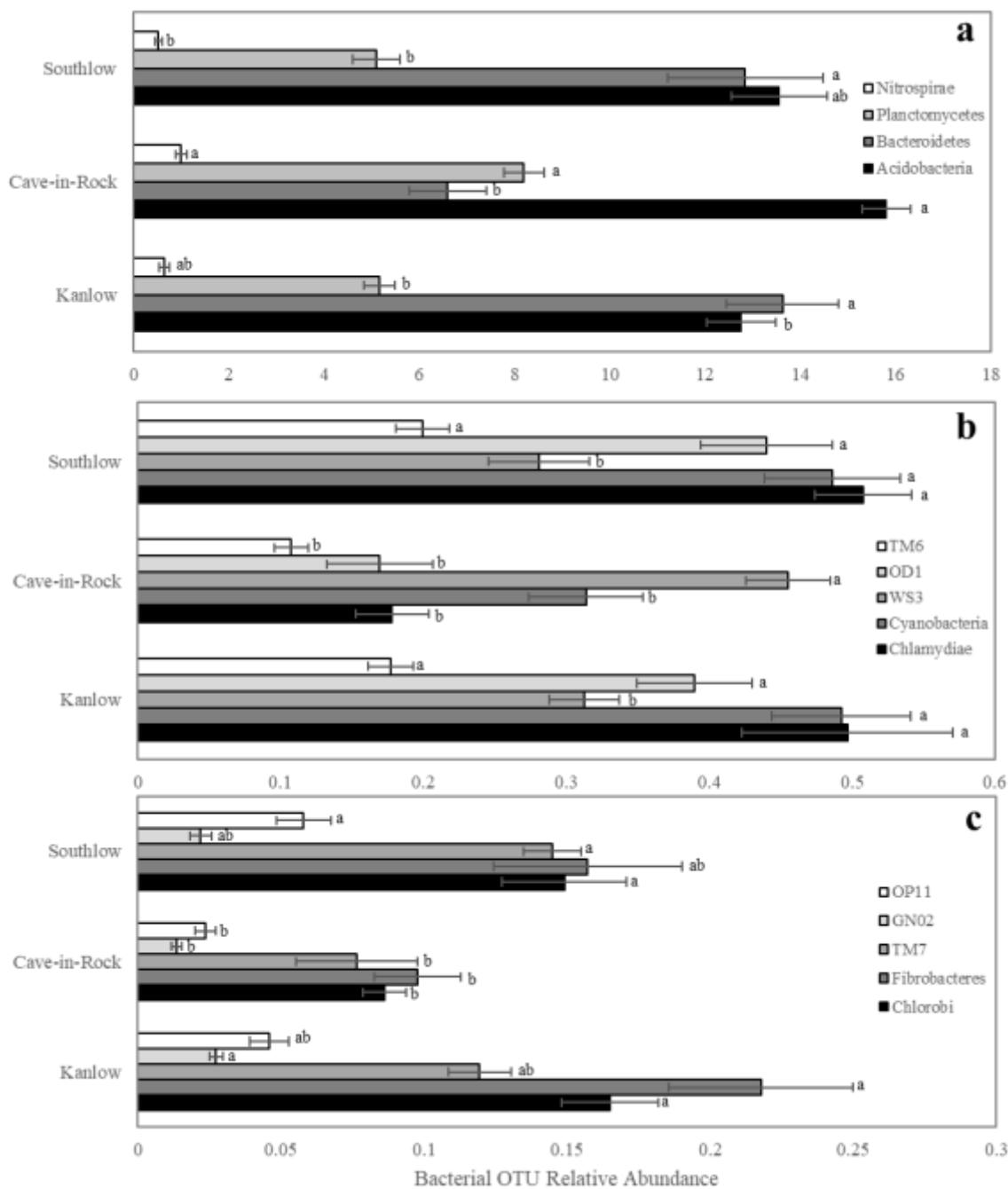


**Figure 3** Non-metric multidimensional scaling (NMDS) of (a) 16S microbial  $\beta$ -diversity (weighted UniFrac, ellipses are standard deviation, stress: 0.167644, n=6), and (b) OTU species relative abundance (ellipses are standard deviation, stress: 0.2287912, n=6) .

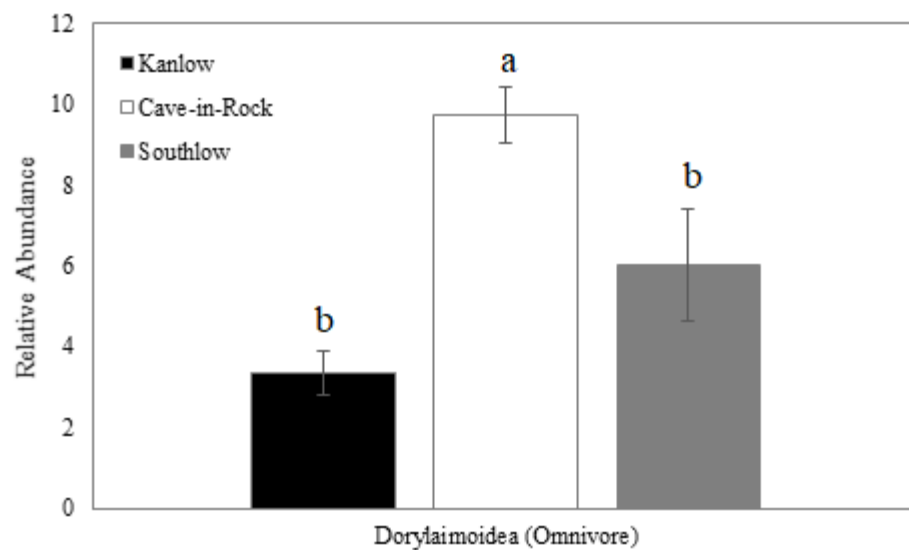


**Figure 4** Bacterial OTU relative abundance significantly impacted by N fertilizer (bars are means  $\pm$  SE, n=3).

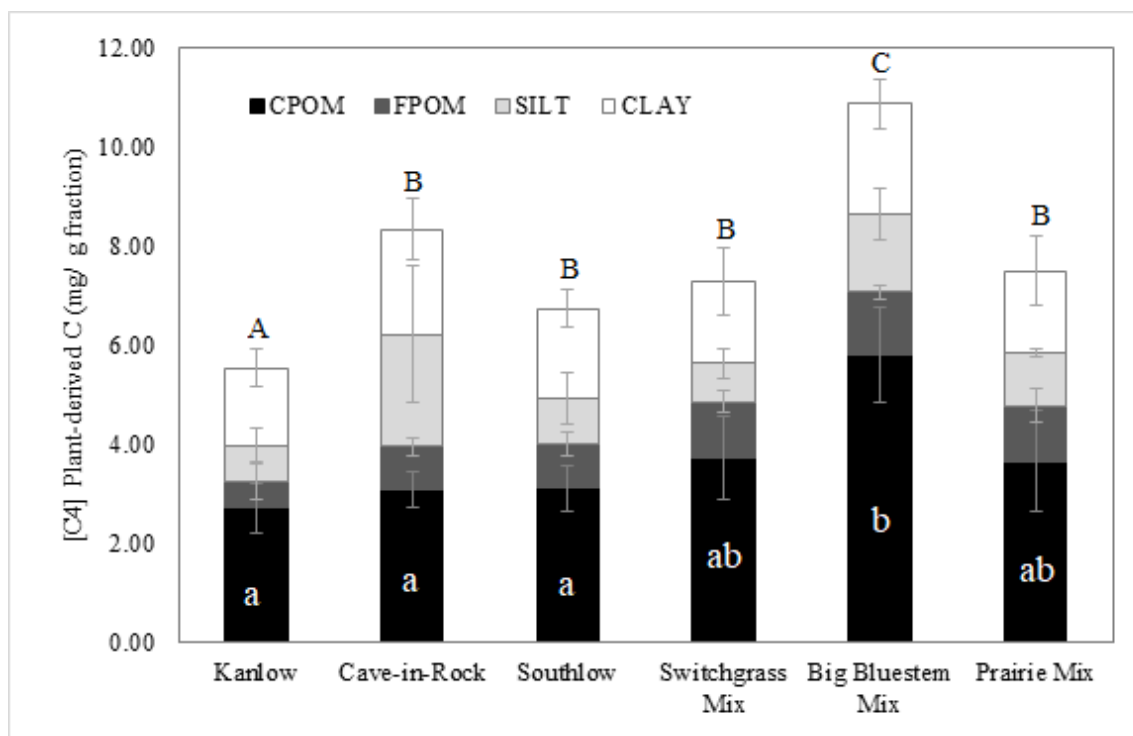




**Figure 5** (a,b,c) Bacterial OTU relative abundance significantly impacted by switchgrass monoculture (n=3). Letters above the bars are significance based on comparisons between switchgrass cultivar treatment.



**Figure 6** Relative abundance of nematode superfamily Dorylaimoidea significantly impacted by switchgrass cultivar monoculture (n=3).



**Figure 7** Plant (C<sub>4</sub>) derived C content normalized to the weight of soil fraction (%) in 1 g bulk soil (bars are means  $\pm$  SE, n=3). Lowercase letters represent Tukey HSD post-hoc for CPOM, uppercase letters represent Tukey HSD post-hoc for BULK soil.

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